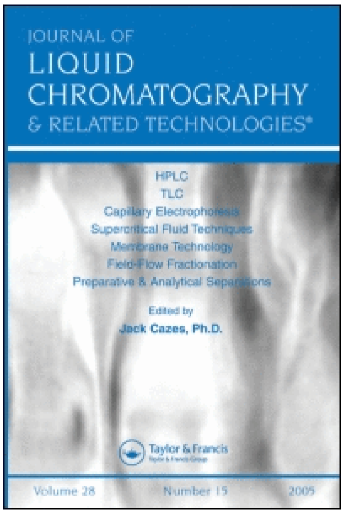

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DIRECT LIQUID CHROMATOGRAPHIC SEPARATION OF 3R-TRANS, 3S-CIS, AND 3R-CIS 1,1-DIMETHYLETHYL (4R-CIS)-6-CYANOMETHYL-2,2-DIMETHYL-1,3-DIOXANE-4-ACETATE ENANTIOMERS ON A CELLULOSE TRIS (3,5-DIMETHYLPHENYL CARBAMATE) CHIRAL COLUMN

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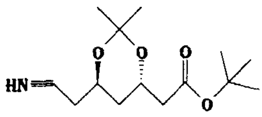
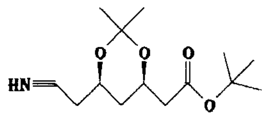
ABSTRACT

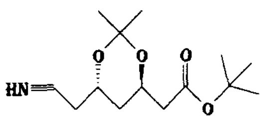
Direct liquid chromatographic separation of 3R-trans, 3S-cis and 3R-cis 1,1-dimethylethyl (4R-cis)-6-cyanomethyl-2,2-dimethyl-1,3-dioxane-4-acetate enantiomers on a cellulose tris (3,5-dimethylphenyl carbamate) column is described. The 3R-cis isomer is an intermediate in the synthesis of atorvastatin (a HMG-CoA reductase inhibitor). The detection limit for each of the undesired 3R-trans and 3S-cis isomers was 0.2% by area percent normalization. The separation of the undesired 3S-trans diastereomer was occasionally observed, and was found to be greatly affected by slight differences in mobile phase composition.



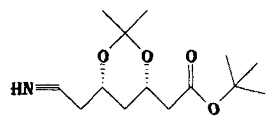
3S-trans



3R-cis



3R-trans



3S-cis

Figure 1. Structures of the isomers.

INTRODUCTION

1,1-Dimethylethyl (4R-cis)-6-cyanomethyl-2,2-dimethyl-1,3-dioxane-4-acetate (3R-cis) is a key intermediate in the convergent synthesis of atorvastatin.[1,2] This ethical pharmaceutical has been shown, in human clinical trials, to significantly reduce serum cholesterol levels by inhibiting the enzyme HMG-CoA reductase.[3,4] In order to control the level of undesired stereoisomers in the bulk pharmaceutical product, a chiral assay for the 3R-cis intermediate was developed. This was done since this intermediate contains all of the chiral centers present in the atorvastatin molecule, and thus, the chiral purity of the final bulk pharmaceutical is determined by the chiral purity of the 3R-cis intermediate.

A cellulose tris (3,5-dimethylphenyl carbamate) chiral column was used to obtain the separation of the undesired 3R-trans, 3S-cis and occasionally the 3S-trans isomers, from the 3R-cis isomer (Figure 1). The effect of eluent composition (hexane: isopropanol ratio, trifluoroacetic acid content), flow rate, and column temperature on the separation of the 3R-trans, 3S-cis, 3R-cis, and 3S-trans isomers was investigated.

EXPERIMENTAL

Apparatus

The HPLC system was composed of the following: a Hitachi L-6200 pump, an Alcott 728 autosampler with a 20μL injector loop, a Hitachi L-4200H detector, a Waters TCM column heater, and a Hitachi D-2500 chromato-integrator. The column used was a Chiracel OD-H, 5 micron, 250 x 4.6mm ID from Chiral Technologies Inc, Exton, PA.

Reagents

HPLC grade hexane(s) and isopropanol were purchased from EM Science, Gibbstown, NJ. All 1,1-dimethylethyl (4R-cis)-6-cyanomethyl-2,2-dimethyl-1,3-dioxane-4-acetate isomers were synthesized in the Chemical Development Department of the Parke-Davis Pharmaceutical Research Division, Holland, MI.

Chromatographic Conditions

The mobile phase used consisted of 97 parts hexane, and 3 parts isopropanol (mixed v/v). The column temperature was 30°C (except for the temperature study). The ultraviolet detection wavelength was 215 nanometers. Flow rate was 0.8 mL/min (except for the flow rate study). The amount of sample injected for the determination of chiral purity was about 200 μg (in a 20μL injection volume).

RESULTS AND DISCUSSION

The hexane/isopropanol mobile phase used in this system resulted in the separation of the 3R-trans, 3S-cis and 3R-cis isomers. Isopropanol content, flow rate, and column temperature were varied to optimize the separation of the enantiomers while maintaining a low detection limit.

Table 1 and Figure 2 show the results of varying the isopropanol content in the mobile phase from 1 to 7% (v/v). The isomers resolution from each other increased with decreased isopropanol content.

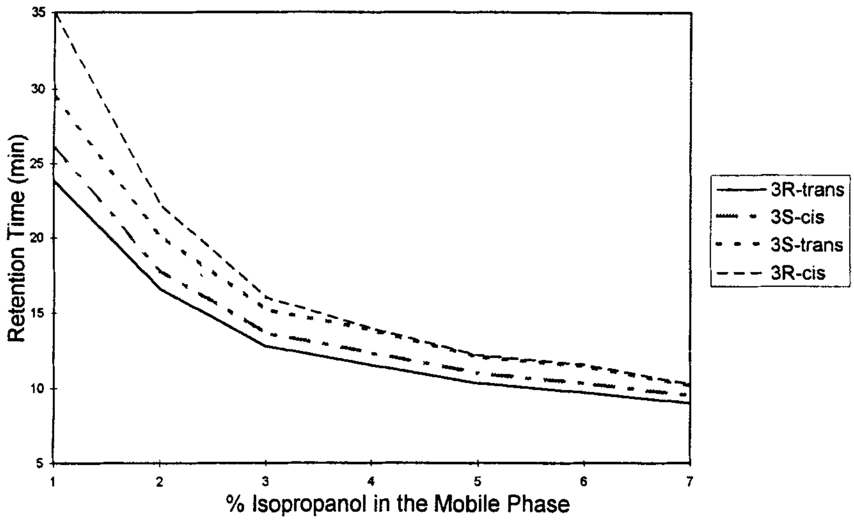


Figure 2. Graph of isopropanol content versus isomer retention time.

Table 1

Resolution Resulting from Alterations in the Hexane/Isopropanol Ratio of the Mobile Phase

Hexane IPA Ratio	Resolution 3R-trans, 3S-cis	Resolution 3S-cis, 3S-trans	Resolution 3S-trans, 3R-cis	Resolution 3S-cis, 3R-trans
99.1	1.89	2.14	2.99	----
98.2	1.44	2.35	1.82	----
97.3	1.46	2.13	0.89	----
96.4	1.51	Coeluted	----	2.34
95.5	1.39	Coeluted	----	1.96
94.6	1.31	Coeluted	----	2.17
93.7	1.33	Coeluted	----	1.39

Although all four isomers were separated when the mobile phase contained 1 and 2 % isopropanol, the peaks were broadened due to longer retention on the column. This resulted in a loss of the desired detection level of 0.2%.

Table 2

Resolution and Retention Times of the Isomers at Variable Flow Rates

	0.6 mL/min	0.8 mL/min	1.0 mL/min
Retention 3R-trans (min)	17.56	12.78	10.56
Retention 3S-cis (min)	18.80	13.67	11.30
Retention 3S-trans (min)	20.99	15.22	12.58
Retention 3R-cis (min)	22.54	16.04	13.26
Resolution 3R-trans, 3S-cis	1.50	1.46	1.33
Resolution 3S-cis, 3S-trans	2.11	2.13	1.86
Resolution 3S-trans, 3R-cis	1.20	0.89	0.80

Table 3

Resolution and Retention Times of the Isomers at Different Column Temperatures

	25°C	30°C	35°C
Retention 3R-trans (min)	14.39	12.78	12.19
Retention 3S-cis (min)	15.36	13.67	13.08
Retention 3S-trans (min)	17.43	15.22	14.32
Retention 3R-cis (min)	19.18	16.04	15.26
Resolution 3R-trans, 3S-cis	1.32	1.46	1.48
Resolution 3S-cis, 3S-trans	2.22	2.13	1.58
Resolution 3S-trans, 3R-cis	1.64	0.89	1.16

The mobile phase flow rate was varied from 0.6 mL/min to 1.0 mL/min. Resolution was slightly increased with a lower flow rate (Table 2). The detection limit remained acceptable at the lower flow rate of 0.6 mL/min.

The column temperature was varied from 25°C to 35°C, and this had some effect on the resolution of the isomers. As temperature was decreased the resolution slightly increased. The results are shown in Table 3. Trifluoroacetic acid was added to the mobile phase at the 0.1% level (v/v). This modifier had no effect on the separation (data not shown).

Table 4

Mobile Phase Aging Experiment Testing the Resolution of the 3S-Trans Diastereomer From the 3R-Cis Intermediate Over Time

Time	0 Days	1 Day	3 Days	8 Days	13 Days
Retention 3R-trans (min)	12.78	12.70	12.72	13.44	13.47
Retention 3S-cis (min)	13.67	13.59	13.63	14.38	14.40
Retention 3S-trans (min)	15.22	15.86	15.16	16.18	16.16
Retention 3R-cis (min)	16.12	15.86	16.00	17.36	17.72
Resolution 3R-trans, 3S-cis	1.46	1.49	1.52	1.42	1.38
Resolution 3S-cis, 3S-trans	2.13	2.08	2.07	2.17	2.17
Resolution 3S-trans, 3R-cis	0.89	0.76	0.90	1.11	1.50

The resolution of 3R-trans, 3S-cis, and 3R-cis from each other on the HPLC system was consistent using the parameters in the chromatographic conditions section. These conditions achieved separation of the above three isomers, while retaining the ability to detect them at low levels. It was observed that the fourth isomer 3S-trans coeluted with the 3R-cis intermediate in most of the above experiments. If some separation occurred, the low detection limit was lost. While investigating the method before validation, mobile phase was used which had been prepared a month earlier. All four isomers were well resolved from each other. When new mobile phase was prepared, the resolution of the 3S-trans isomer from the 3R-cis intermediate was lost. After much investigation, it was determined that as time passed after mobile phase preparation, resolution of the 3S-trans isomer from the 3R-cis intermediate on the HPLC system was improved. The rate of this 'aging' phenomenon varied depending on the brand (JT Baker and Fisher), purity, or age of the hexane used. This observation was investigated to determine if the 3S-trans isomer could be separated from the 3R-cis intermediate consistently.

An experiment was done to show the 'aging' phenomenon. Mobile phase was prepared and the resolution of all four isomers was measured after preparation, and on the following days. The results are shown in Table 4 and Figure 3.

These results show that the changes in mobile phase, with time, were not caused by a decrease in the hexane:isopropanol ratio due to evaporation. If this were the case, retention times would get progressively earlier; however, elution occurred later after 2 weeks.

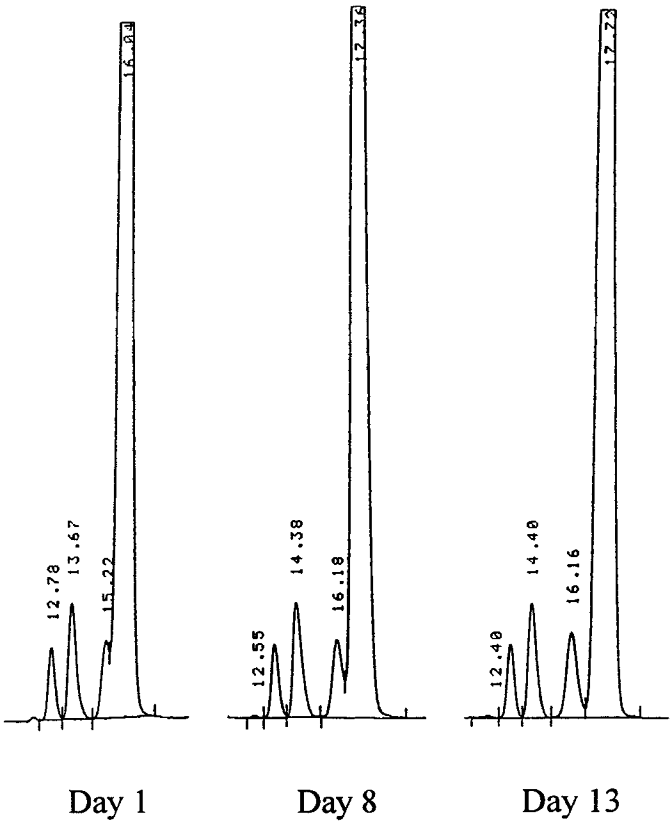


Figure 3. Mobile phase 'aging' experiment.

Water content in the mobile phase was varied (from 0.01-0.9%) and showed no effect in the resolution of 3S-trans and 3R-cis isomers. Mobile phase (which had been 'aged') was tested for peroxide formation,[6] because it has been reported that hydroperoxides may form by the oxidation of hydrocarbons in the presence of dissolved oxygen.[5] No peroxides were detected.

Air was bubbled through both the hexane, before mobile phase preparation, and the mobile phase itself. This was done as an attempt to speed the 'aging' process by exposure to oxygen in the air. The resulting mobile phases did not separate the 3S-trans isomer from the 3R-cis intermediate.

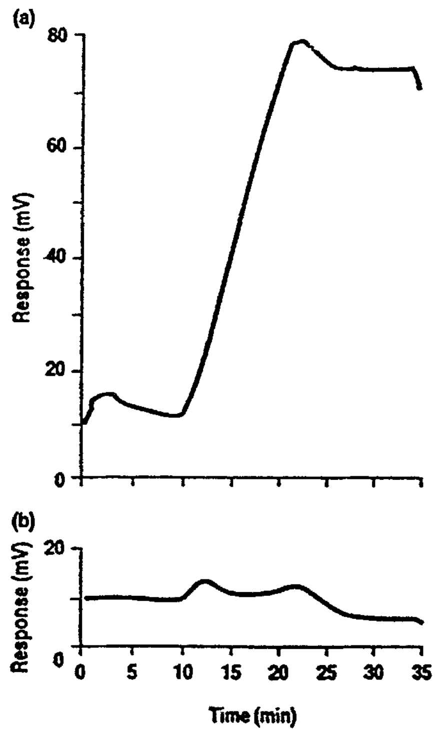


Figure 4. Blank gradient baselines for mobile phases containing (a) year old hexane and (b) fresh hexane.[7]

An experiment was done to test the affects of light exposure on the mobile phase. Mobile phase was prepared, then half was stored in the dark and half in the light. Both were tested at intervals over two weeks time. They 'aged' at the same rate showing that light had no effect on the 'aging' process (data not shown).

Problems with hexane/isopropanol mobile phase over time in normal phase HPLC have been previously reported.[7] Specifically, T. Michnik and D. Federighi of Cell Therapeutics, Inc.,[7] generated two different blank gradient runs using their own method. The first, used one year old hexane in the mobile phase, and the second, three month old hexane. These are shown in Figure 4.[7] The 'age' of hexane can have some effect on normal phase HPLC chromatography; however, the explanation for the change over time is uncertain.

CONCLUSIONS

This method is effective in determining the chiral purity of the 3R-cis intermediate in the synthesis of atorvastatin. It has been routinely used to detect the undesired 3S-cis enantiomer down to the 0.2% level. The 3R-trans isomer is nicely separated, but is not of concern, because it is not detected in the intermediate.

Although the separation of the undesired 3S-trans diastereomer was observed using 'aged' mobile phase, the reason for this is not understood, limiting reproducibility. Another method was, therefore, developed to quantify the 3S-trans isomer.

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